PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/70, 31/57 // (A61K 31/70 A61K 31/57) (A61K 31/57 A61K 31/19)

A1

(11) International Publication Number: WO 90/04399

(43) International Publication Date: 3 May 1990 (03.05.90)

(21) International Application Number: PCT/GB89/01266

(22) International Filing Date: 23 October 1989 (23.10.89)

(30) Priority data:
8824835.6 24 October 1988 (24.10.88) GB

(71) Applicant *(for all designated States except US):* THE UNIVERSITY COURT OF THE UNIVERSITY OF EDINBURGH [GB/GB]; South Bridge, Edinburgh EH8 9YL (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only) :* EDWARDS, Christopher, Richard, Watkin [GB/GB]; Department of Medicine, Western General Hospital, Edinburgh EH4 2XU (GB).

(74) Agent: HALE, Stephen, Geoffrey; J.Y. & G.W. Johnson, Furnival House, 14-18 High Holborn, London WC1V 6DE (GB).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ADMINISTRATION OF CORTICOSTEROIDS

(57) Abstract

The effect of an 11-betahydroxy corticosteroid at the site of its desired action on a patient can be potentiated by the use of an inhibitor for the patient's 11-betahydroxy steroid dehydrogenase. The active components of liquorice, principally glycyrrhizic acid and glycyrrhetinic acid and their derivatives, have this potentiating effect if they are administered in association with the 11-betahydroxy corticosteroid, e.g. hydrocortisone, for example as a pharmaceutical composition.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Fasso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	HU	Hungary	NO	Norway
BJ	Benin	IT	Italy	RO	Romania
BR	Brazil	JP	Japan	SD	Sudan
CA	Canada	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic			SN	Senegal
CG	Congo	KR	Republic of Korea	SU	Soviet Union
CH	Switzerland	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany, Federal Republic of	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

ADMINISTRATION OF CORTICOSTEROIDS

Field of the invention

The present invention relates to the administration of corticosteroids to human and animal patients.

Background of the invention

Corticosteroids such as cortisol are administered to patients for physiological replacement therapy and for their anti-inflammatory effect. The active form of these steroids is the 11-betahydroxy form. However, there is a tendency for the administered 11-betahydroxy steroid to be deactivated after administration by conversion in tissues such as the liver and kidney, to the corresponding 11-keto steroid, e.g. cortisol is converted to cortisone. In order to decrease or eliminate such conversion the 11-betahydroxy steroids administered for anti-inflammatory effect have been modified, primarily by addition of a fluorine substituent at the 9-position of the steroid nucleus. However, the enhanced potency of these 9-alpha-fluorinated corticosteroids, whilst being an advantage for therapy, is associated with more marked suppression of the hypothalamic-pituitary-adrenal axis. This can be a serious problem in patients given topical steroid therapy. It would thus be an advantage if it was possible to potentiate the effect of a corticosteroid at the site of the desired action without having to have a steroid structure that was designed to delay its systemic metabolism.

It is known that conversion of cortisol to cortisone in humans is catalysed by 11-betahydroxysteroid dehydrogenase, a microsomal enzyme complex consisting of 11-betadehydrogenase converting cortisol to cortisone and 11-oxoreductase converting cortisone to cortisol (Endocrinology 1985:116:552-560). A similarly named enzyme in the rat catalyses conversion of corticosterone

into 11-dehydro-corticosterone.

It has been disclosed in The Lancet, 10 October 1987, 821-824, that administration of liquorice, the active components of which are glycyrrhizic acid and its hydrolytic product, glycyrrhetinic acid, to normal adult human subjects in amounts of 200 g/day causes an increase in urinary free cortisol and has other effects characteristic of 11-betahydroxysteroid dehydrogenase deficiency indicating inhibition of 11-betahydroxysteroid dehydrogenase by administration of liquorice.

Summary of the invention

The present invention seeks to utilise that discovery in the administration of corticosteroids. We have found that the effect of a corticosteroid at the site of the desired action can be potentiated by locally inhibiting the conversion of the 11-betahydroxysteroid into its inactive form.

According to the invention an inhibitor for 11-beta-hydroxysteroid dehydrogenase is administered to a patient in association with administration of an 11-betahydroxy-steroid capable of being metabolised by the 11-betahydroxy-steroid dehydrogenase.

The administration may take place shortly before (e.g. 60 minutes before) or shortly after administration of the 11-betahydroxy steroid or simultaneously with it.

Thus, the invention includes a pharmaceutical composition for administration to a patient, comprising an 11-betahydroxy corticosteroid and an inhibitor for the patient's 11-betahydroxysteroid dehydrogenase.

Compounds having aldosterone-like actions, for example carbenoxolone (3β-(3-carboxypropionyloxy)-11-oxo-olean-12-

en-30-oic acid) and its physiologically acceptable salts such as the sodium salt, may be used to potentiate the effect of the corticosteroid. Preferably, however the material used is a liquorice product, especially glycyrrhizic acid or glycyrrhetinic acid or a physiologically acceptable derivative thereof such as an alkali metal or alkaline earth metal salt. Glycyrrhizic acid and its salts are preferred in compositions intended for internal administration owing to higher water-solubility but they are converted in vivo to glycyrrhetinic acid or its salts which are more effective in their inhibiting action.

The 11-betahydroxy corticosteroids administered may be any of those previously proposed for administration, for example hydrocortisone (cortisol). Because of the inhibition of the 11-betahydroxysteroid dehydrogenase it is not necessary to use the stronger corticosteroids having adverse side effects such as those bearing a fluorine substituent at the 9-position and they are preferably avoided.

The corticosteroid and inhibitor may be administered in any convenient way, externally (topically) or internally by injection (e.g. into a joint), or by mouth (e.g. by inhalation). The steroid may be administered in conventional amounts, for example topical application of an ointment, cream or the like containing about 0.5% by weight hydrocortisone, and the inhibitor may be included in any amount sufficient to achieve significant inhibition of the 11-betahydroxysteroid dehydrogenase, for example a concentration of about 2% by weight of glycyrrhetinic acid or a derivative in such an ointment or cream. We have found that whereas an ointment or cream containing hydrocortisone normally has little effect on the skin owing to the high concentration of 11-betahydroxysteroid dehydrogenase there, the inclusion of glycyrrhetinic acid in the ointment or cream causes the ointment or cream to have a significant effect even at a hydrocortisone dilution of 1 in 100,000.

The formulations administered may contain conventional pharmaceutical excipients or diluents and may be suitably packaged. If the two ingredients are not administered in a single composition a pack may comprise separate but associated compositions each containing one of the two active ingredients and designed to be administered in association with the other.

In vitro studies have shown that the mineralocorticoid receptor is non-specific and does not distinguish between aldosterone and cortisol. In vivo certain tissues with this receptor are aldosterone selective (e.g. kidney and parotid) whereas others with the same receptor are not (e.g. hippocampus, heart). Studies in rats using immunohistochemistry and in vitro incubation have shown that the enzyme 11β-hydroxysteroid dehydrogenase (11β-OHSD) is present in the aldosterone-selective tissues in much higher concentrations than in those which are non-selective. The localisation in the selective tissues is such that it is ideally situated to act as either a paracrine or possibly autocrine mechanism protecting the receptor from exposure to corticosterone (and therefore presumably cortisol in man) and hence determining its apparent specificity. Autoradiography experiments confirmed that inhibition of the enzyme results in loss of protection. Under these conditions ^{3}H-corticosterone was bound to similar sites as was ^{3}H-aldosterone. These findings appear to explain why patients with congenital deficiency of 11β-OHSD or those in whom the enzyme has been inhibited by liquorice develop sodium retention, hypokalemia and hypertension.

Detailed disclosure

In vitro experiments with either cytosolic preparations of the mineralocorticoid (type 1) receptor or where the cloned receptor has been expressed in transfected cells

have shown that its affinity is similar for aldosterone, cortisol, corticosterone and deoxycorticosterone. These contrast with *in vivo* studies in which these type I receptors in the kidney, parotid and colon are aldosterone-selective, whereas those in the hippocampus do not distinguish between aldosterone and corticosterone. These results led to a suggestion that there must be a factor other than the receptor responsible for determining the aldosterone tissue specificity, perhaps extravascular corticosteroid binding globulin (CBG) which preferentially bound cortisol or corticosterone (the major glucocorticoid in the rat). However, recent studies in the 10 day old rat which has very low levels of CBG showed that the *in vivo* specificity of aldosterone was maintained despite the much higher levels of corticosterone.

11β-OHSD is the microsomal enzyme complex responsible for the interconversion of cortisol and cortisone. Recent work has shown that it consists of two separate enzymes, one converting cortisol to cortisone (11β-dehydrogenase) and the other cortisone to cortisol (11-oxoreductase). Congenital deficiency of the dehydrogenase enzyme is associated with severe hypertension, hypokalemia and suppression of plasma aldosterone and plasma renin activity. This has been called the syndrome of apparent mineralocorticoid excess. Our studies in the first adult to be found to have the syndrome suggested that cortisol was acting as a mineralocorticoid. These were in keeping with other results. We put forward the hypothesis that the normal kidney used 11β-OHSD to convert cortisol to the inactive steroid cortisone and was thus protected from this effect. If this was the case then inhibition of 11β-OHSD would result in a failure of this protective mechanism and thus allow access of cortisol to the non-specific renal mineralocorticoid receptors, resulting in sodium retention. We then found that the active component of liquorice (glycyrrhetinic acid) was a potent inhibitor of 11β-OHSD and proposed that this was the explanation for the sodium-

retaining and potassium-losing actions of liquorice. This resolved the problem as to why liquorice did not have these effects in patients with severe adrenocortical insufficiency or following bilateral adrenalectomy.

11-ß-OHSD has been shown to be present in the liver, kidney, gonads, placenta, lung and intestinal mucosa. The recent purification of 11ß-OHSD has allowed the production of a specific antiserum and hence precise tissue localisation.

We have sought to make a further examination of the tissue distribution of 11ß-OHSD to determine whether the enzyme is appropriately situated to act as either a paracrine or autocrine protector of the mineralocorticoid receptor in aldosterone-selective organs. (In the usual paracrine system a hormone produced by one cell type acts on adjacent cells as compared to an autocrine mechanism where the hormone is produced by and acts on the cell of origin. Here the term paracrine is used to denote the metabolism of the steroid by 11ß-OHSD in cells which do not contain the mineralocorticoid receptor but which can influence the hormonal environment of other cells with the receptor. This contrasts with an autocrine system in which the enzyme and the receptor are in the same cells.) In addition we have looked at tissues which are not aldosterone-selective but which have mineralocorticoid receptors to determine whether this non-selectivity could be explained by an absence of 11ß-OHSD. Finally we have examined the effect of inhibiting 11ß-OHSD on the specific binding of corticosterone by the kidney to determine whether this would result in corticosterone binding in the same sites as aldosterone.

Methodology

A i) Preparation of tissue for enzyme activity

Renal cortex, parotid, heart and hippocampus was

obtained from 10 day old male Sprague-Dawley rats and 0.5g wet weight tissue homogenised in 10ml Krebs Ringer buffer with a Dounce tissue grinder for 30 strokes. Using a fixed mg protein/g wet weight tissue (Bio-Rad protein assay kit) 400µl of a diluted homogenate preparation was incubated at 37°C for 60 mins with 600µl Krebs Ringer buffer (+ 0.2% glucose, 0.2% bovine serum albumin) and 1.2×10^{-8}M ^{3}H-corticosterone (sp. activity 84 Ci/mmol, Amersham International). Following centrifugation, steroids were extracted from the supernatant using ethyl acetate and ^{3}H-corticosterone separated from ^{3}H-11-dehydrocorticosterone using thin-layer chromatography. The percentage conversion of corticosterone to 11-dehydrocorticosterone by 11β-OHSD was then calculated.

ii) Isolation of rat kidney cortical tubules enriched in proximal and distal segments using a Ficoll gradient

2g wet weight of renal cortical tissue was taken from 3 month old male Sprague-Dawley rats and tubules prepared using a mechanically dispersed enzyme solution (0.05% (w/v) collagenase, 0.1% (w/v) hyaluronidase). Following filtration (to remove glomeruli) and successive washing procedures the tubules were subjected to unit gravity sedimentation through a Ficoll gradient. From this both a distal enriched fraction (narrow, transparent tubules) and a proximal enriched fraction (yellowish, broad tubules) were established with purities of 85% and 70% respectively. Tubular integrity was evaluated by the dye exclusion method. A tubular count was made using a haemocytometer and 400µl of each tubular preparation (containing approximately 10^{5} tubules) incubated with 600µl Krebs Ringer buffer (containing 0.2% glucose, 0.2% BSA) and 1.2×10^{-8}M ^{3}H-cortisol. Steroids were extracted and separated as above and 11β-OHSD activity calculated in proximal and distal tubules as % conversion of cortisol to cortisone.

B Immunohistochemical localisation of enzyme activity with a specific antibody 11ß-dehydrogenase

11ß-dehydrogenase was purified to apparent homogeneity from a rat hepatic microsomal preparation. 800-fold purification was achieved using agarose-NADP affinity chromatography. The purified enzyme was a glycoprotein (mw 34000) and had no reductase activity. Antibodies to this homogeneous 11ß-dehydrogenase were raised in female New Zealand white rabbits. Pre-immune and immune sera were stored as 1ml aliquots at -20°C. Three month old male Sprague-Dawley rats (Charles River, Kent) were sacrificed and the kidneys, parotid, heart and hippocampus removed, sliced longitudinally and placed in Bouins fixative for 24 hrs. They were then processed through to paraffin block and sections cut at 4µm. Immunostaining was accomplished using the Avidin-Biotin-Peroxidase method. Reagents used were the Vector "Elite" (Vector Laboratories Inc, Burlingname, California, USA).

C Effect of inhibition of 11ß-OHSD on the autoradiographic localisation of ^{3}H-corticosterone in the kidney

^{3}H-aldosterone (100µCi/100g body weight) or ^{3}H-corticosterone (100 µCi/100g body weight) was given to adult Wistar rats via a jugular vein cannula 1 hour before sacrifice. In a separate experiment 5mg glycyrrhizic acid was given subcutaneously 60 minutes before the ^{3}H-corticosterone to inhibit 11ß-OHSD. In a further experiment 1mg unlabelled corticosterone was given subcutaneously 30 minutes prior to the ^{3}H-corticosterone to determine the non-specific binding. After sacrifice kidneys were removed, frozen, cryostat sectioned (25µ thickness) and then exposed to ^{3}H-ultrofilm for two weeks.

Results

The activity of 11β-OHSD in the homogenates of renal cortex, parotid, hippocampus and heart as determined by % conversion of ^{3}H-corticosterone to ^{3}H-11-dehydrocorticosterone was compared. The highest level of activity was present in the kidney (35 ± 2%) with lower levels in the parotid (10 ± 2%). Little or no enzyme was present in the hippocampus (1 ± 1%) or heart.

Density gradient separation showed that both the proximal and the distal tubular preparations were capable of converting cortisol to cortisone, % conversion of ^{3}H-cortisol to ^{3}H-cortisone being 33% and 23% respectively after 60 minutes and 18% and 15% respectively after 15 minutes. In the three experiments performed enzyme activity was higher in the distal than in the proximal tubule.

Immunohistochemistry confirmed the high level of enzyme present within the kidney. In contrast to the density gradient separation the enzyme was mainly in the proximal tubule and not in the distal nephron. However the enzyme appeared also to be localised either in or immediately adjacent to the vasa recta alongside the papillary collecting tubules.

In the parotid 11β-OHSD was present in both the intercalated and striated ducts but was not found in the acini. No localised enzyme was present in the heart or hippocampus. Autoradiography with ^{3}H-aldosterone showed the expected binding in the cortex-outer medulla and papilla-inner medulla. In contrast the uptake of ^{3}H-corticosterone in these sites was very low and little different from the non-specific binding. However, after inhibition of 11β-OHSD the pattern of ^{3}H-corticosterone binding was markedly changed and was now similar to that with ^{3}H-aldosterone.

The above results show that two major aldosterone-selective tissues (kidney and parotid) have much higher levels of 11β-OHSD than those organs with the same mineralocorticoid receptor but which are not aldosterone specific (heart and hippocampus). The position of enzyme in the kidney as assessed by immunohistochemistry (proximal tubule and vasa recta) suggests that the enzyme is situated in a position which would allow it to act as a paracrine protector of the type 1 receptor in the cortical and papillary collecting tubule. The localisation of this receptor in the rat has been demonstrated using tritiated aldosterone binding. This was found in both the renal cortex-outer medulla and papilla-inner medulla. These results were in keeping with those obtained by others who measured aldosterone binding along the nephron and showed high levels of nuclear labelling in the cortical collecting tubule. The density gradient studies confirmed the presence of enzyme activity in the proximal tubule but also suggested that there was conversion of cortisol to cortisone by the distal nephron. One possible explanation for these apparently discrepant results is that there may be a different 11β-dehydrogenase in the distal tubule.

The free (non-protein bound) fraction of cortisol in plasma is filtered by the kidney; 80-90% of this is reabsorbed passively by the tubule and only about 0.5% is excreted unchanged in the urine. If this reabsorption is by the proximal tubule then this would allow metabolism of cortisol to cortisone. In addition there would be a need for cortisol which is not filtered to be metabolised. This could be by diffusion from the peritubular capillaries into the proximal tubular cell. It has been suggested that 11β-OHSD was involved in the cellular capture mechanism for cortisol and that, at least in the kidney, oxidation to cortisone was apparently a prerequisite for cellular release of the steroid. By way of contrast aldosterone which is not significantly metabolised by 11β-OHSD would be reabsorbed and pass into the peritubular plexus without

inactivation. Aldosterone and the inactive glucocorticoid metabolite would then pass down the peritubular plexus to reach the collecting tubule where aldosterone diffuses across the basolateral cell membrane to gain access to the cytoplasmic or possibly intranuclear receptor.

The blood supply to the renal medulla suggests that there would probably be a need for a different localisation of 11β-OHSD to prevent access of cortisol to the papillary collecting tubule. Some vasa recta arise directly from interlobular arteries and do not have an initial circulation through a glomerular tuft. In this case the enzyme would need to be in close relation to the descending vasa recta. Our immunohistochemistry results would be in keeping with this.

A crucial test of our paracrine hypothesis is the demonstration that inhibition of 11β-OHSD results in loss of the selectivity of binding of aldosterone in tissues such as the kidney. The autoradiography results strongly suggest that this is the case. When the enzyme is intact corticosterone (the steroid equivalent to cortisol in man) is not taken up by the kidney. However, when 11β-OHSD is inhibited by glycyrrhizic acid (one of the components of liquorice which is hydrolysed <u>in vivo</u> to the major active component glycyrrhetinic acid) the aldosterone selectivity is lost and corticosterone now binds in a distribution which is similar to that of aldosterone. It remains to be determined whether all the corticosterone uptake is by the same receptors as those binding aldosterone. Using isolated tubules it has been shown that corticosterone binding sites were concentrated in the cortical collecting tubule. Part but not all of this steroid could be displaced by unlabelled aldosterone. In these studies corticosterone had direct access to the nephron from the incubation medium and was thus able to bypass the protective moat provided by 11β-OHSD.

In the parotid the enzyme was found in both the intercalated and striated ducts but not in the acini. Previous studies using micropuncture have shown that sodium reabsorption and potassium secretion occurs in the striated but not in the intercalated ducts in rat submaxillary glands. This is in keeping with the salivary gland localisation of $(Na^+ + K^+)$-ATPase in the cat as assessed using ^{3}H-ouabain where the cells of the striated duct were heavily labelled. These results would suggest that the type 1 receptor is likely to be in the striated duct.

It is not clear how mineralocorticoids gain access to the striated duct. The blood supply to the parotid is very different to that in the kidney. The arteries accompany the ducts within the lobules and break up into capillary and precapillary plexuses around the striated ducts. Arteriolar arcades then arise which continue to supply the acini. Blood flow studies have suggested that the flow was mainly countercurrent to that of saliva. If aldosterone then entered via the basal cell membrane of the striated duct then 11ß-OHSD would need to be present within the cells of the duct. This appears to be the case and would be in keeping with an autocrine system. If however aldosterone enters via the luminal membrane then this would allow the possibility of upstream metabolism by the enzyme in the intercalated duct.

The very low levels of the enzyme in the hippocampus and the heart associated with the lack of any specific tissue localisation suggest that these tissues contain no 11ß-OHSD mechanism for the inactivation of cortisol or corticosterone. This would allow direct access of these steroids to the receptor and would thus be in keeping with previous work showing that there are type 1 receptors in these tissues but they are not aldosterone-selective. In fact, behavioural and biochemical studies in the rat have revealed a number of responses that are under stringent

control of corticosterone acting via the limbic type I receptor. Aldosterone appeared a competitive antagonist in these studies.

Human 11β-OHSD has yet to be purified and hence no antisera have been produced. It would seem likely, based on measurement of enzyme activity, that the tissue localisation would be similar to that we have found in the rat. The human kidney is known to be an important site for the conversion of cortisol to cortisone. In patients with renal disease one might anticipate that this mechanism would be impaired. We have recently shown that plasma cortisone levels are reduced in such patients and there is a highly significant negative correlation between plasma cortisone and creatinine. It remains to be determined what role this might play in sodium retention in renal failure. It is also possible that the lack of renal 11β-OHSD might be important in delaying the development of hyperkalemia in such patients. These effects would require the loss of enzyme activity to be dissociated from that of the mineralocorticoid receptors in the collecting tubule.

These studies all indicate the importance of this steroid shuttle. Congenital or acquired deficiency of the enzyme converting cortisol to cortisone results in cortisol functioning as a potent mineralocorticoid. We would suggest that without this paracrine mechanism we would be 'pillars of salt'.

Having demonstrated the role that 11β-OHSD plays in protecting the mineralocorticoid receptor we have now begun to examine the possibility that 11β-OHSD may be important in regulating access of cortisol to the glucocorticoid receptor. To do this we have employed the skin vasoconstrictor assay, used to determine the potency of topical corticosteroids. The assay is based on the degree of skin pallor produced by application of corticosteroid to the forearm. Such pallor can be assessed on a visual analogue

scale and has been validated as an accurate measure of glucocorticoid potency and dose-response.

We have examined the effect of 11β-OHSD inhibition by topical glycyrrhetinic acid on hydrocortisone skin vasoconstrictor potency. Twenty five tests were performed on 23 volunteers who had had no previous exposure to exogenous corticosteroids.

Test solutions comprised:-

(i) hydrocortisone acetate alone
(ii) glycyrretinic acid (GE) alone
(iii) hydrocortisone acetate plus GE (single solution)

All solutions were freshly prepared in 95% ethanol in the following concentrations:-
hydrocortisone: 1, 3, 10, 30 and 100 mg/ml
GE : 20 mg/ml (for all tests).

Test substances (10 µl) were applied to 7 mm^2 sites, demarcated with silicone grease, on the flexor aspect of the forearm. After drying, the solutions were occluded with polyester film for 16 h. The film was then removed. The degree of blanching of each test area was assessed 1, 2, 3 and 6 h after removal of the film by 2 observers using a linear analogue scale. Scoring was:-

0 no blanching
1 mild blanching
2 definite blanching
3 intense blanching

All solutions were applied in random sequence and scoring was double blind.

Time-effect curves for each test substance were plotted and the area under the curves calculated. GE alone

(solution ii) had no effect. The results for solutions (i) and (iii) were as follows:-

Hydrocortisone dose mg	Area under curve of skin vasoconstriction	
	Solution (i)	Solution (iii)
1	0.17 ± 0.07	0.56 ± 0.36
3	0.76 ± 0.24	1.46 ± 0.44
10	0.49 ± 0.20	2.82 ± 0.46
30	0.13 ± 0.06	3.51 ± 0.66
100	0.68 ± 0.35	2.35 ± 0.51

The differences at hydrocortisone doses of 10 - 100mg are highly significant ($p < 0.01$).

These results demonstrate unequivocal potentiation of glucocorticoid action by GE. This has important consequences for topical and other targetted glucocorticoid therapy. Using immunohistochemistry we have confirmed that 11ß-OHSD is present in the skin with high concentrations in the epidermis and in a number of dermal structures. Studies on rats, mice and human skin have shown 11ß-OHSD bioactivity.

Very recently we have found that cerebellum, one of the few tissues that lacks mineralocorticoid receptor but has a high concentration of glucocorticoid receptor, expresses 11ß-OHSD mRNA and shows both enzyme bioactivity and immunoreactivity. Other non-mineralocorticoid target tissues showing 11ß-OHSD activity include the testis, liver and lung. In the testis we have shown that the enzyme is in interstitial cells. Of great interest is the observation that, in the rat, the enzyme is not present in testis at birth but appears at about 20 days, coinciding with the onset of puberty. In vitro, corticosteroids block the production of testosterone by interstitial cells. Thus novel 11ß-OHSD activity at puberty may enhance testicular testosterone production by locally inactivating glucocorticoid. This could be a crucial step in regulation of the

onset of puberty.

In the lung there is evidence for both 11ß-dehydrogenase and 11-oxoreductase activity. It has been suggested that increased cortisone to cortisol conversion (oxoreductase) is important in the maturation of the lung, but when using human foetal lung explants as opposed to monolayer cultures only dehydrogenase activity has been found. This suggests that inhibition of 11ß-OHSD could enhance lung maturation by locally increasing glucocorticoid concentrations. Of greater clinical application is the possibility that 11ß-OHSD inhibition might potentiate endogenous or inhaled glucocorticoid therapy in the treatment of asthma, in parallel with the effect of GE on topical glucocorticoid activity. This also raises the possibility that some corticosteroid-resistant cases of disorders that are usually glucocorticoid-sensitive could be, at least in part, due to excessive tissue dehydrogenase activity.

Earlier studies on 11ß-OHSD demonstrated that the liver was an important site both for the conversion of cortisol to cortisone and also for the reverse oxoreductase reaction. The function of this dual activity within the same organ is unknown. However, liquorice extract is extensively used by general practitioners in Japan in the treatment of hepatitis. Apparently, abnormally elevated plasma concentrations of liver enzymes fall on therapy with liquorice but rise again when this is stopped. The effect of liquorice could be due to inhibition of liver dehydrogenase leading to enhanced hepatic cortisol concentrations, providing local suppression of inflammation.

Potentiation of systemic glucocorticoid action might be expected to also increase deleterious side-effects such as hypothalamo-pituitary-adrenal axis suppression. However, in rat pituitary there is little or no 11ß-OHSD activity and enzyme immunoreactivity is low or absent.

Furthermore, our patient with 11β-OHSD deficiency had normal circulating levels of ACTH. This suggests that enzyme inhibitor-mediated glucocorticoid potentiation may not lead to an equal increase of inhibition of the hypothalamo-pituitary-adrenal axis. Thus organ-specific manipulation of glucocorticoid potency might be achieved by topical or systemic 11β-OHSD inhibition.

Claims

1. The use of an inhibitor for 11-betahydroxysteroid dehydrogenase for the preparation of a medicament for potentiating the effect of an 11-betahydroxy corticosteroid at the site of its desired action on a patient.

2. The use of glycyrrhizic acid or glycyrrhetinic acid or a physiologically acceptable derivative thereof for the preparation of a medicament for potentiating the effect of hydrocortisone at the site of its desired action on a human patient.

3. A method of treatment of a patient by administration of an 11-betahydroxy corticosteroid, in which method the local conversion of the 11-betahydroxy corticosteroid into an inactive form catalysed by the 11-betahydroxysteroid dehydrogenase occurring naturally in the patient is inhibited by the administration of an inhibitor for the 11-betahydroxysteroid dehydrogenase to the patient in association with the 11-betahydroxy corticosteroid treatment.

4. A method of treatment of a human patient by administration of hydrocortisone in which method a liquorice product selected from the group consisting of glycyrrhizic acid, glycyrrhetinic acid and derivatives thereof is administered to the patient in association with the hydrocortisone treatment for the local inhibition of 11-beta-hydroxysteroid dehydrogenase.

5. A method as claimed in claim 3 in which the inhibitor is administered between 60 minutes before and shortly after administration of the 11-betahydroxy corticosteroid.

6. A method as claimed in claim 4 in which the

hydrocortisone and the liquorice product are administered essentially simultaneously.

7. A pharmaceutical composition for administration to a patient, comprising an inhibitor for the patient's endogenous 11-betahydroxysteroid dehydrogenase and an 11-betahydroxy corticosteroid capable of being metabolised by the 11-betahydroxysteroid dehydrogenase.

8. A pharmaceutical composition for administration to a human patient comprising hydrocortisone and a liquorice product selected from carbenoxolone, glycyrrhizic acid glycyrrhetinic acid and derivatives thereof.

9. A pharmaceutical composition as claimed in claim 8 in the form of an ointment or cream for topical application.

10. A pharmaceutical composition as claimed in claim 8 in a form suitable for administration internally by injection or by mouth and containing glycyrrhizic acid or carbenoxolone.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/01266

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 31/70, A 61 K 31/57,//(A 61 K 31/70, 31:57), //(A 61 K 31/57, 31:19)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	GB, A, 843135 (BIOREX LAB. LTD) 4 August 1960, see page 3, example 6 --	1,2,7-10
X	FR, M, 4666 (INSTITUT DE RECHERCHE SCIENTIFIQUE IRS) 16 January 1967, see claims 1-3 --	1,2,7-10
X	DE, A, 3443242 (YISSUM RESEARCH DEVELOPMENT CO. OF THE HEBREW UNIVERSITY OF JERUSALEM) 28 May 1986, see page 9, example 3 --	1,2,7-10
A	The Lancet, vol. II, no. 8563, 10 October 1987, The Lancet Ltd, London (GB) P.M. Stewart et al.: "Mineralocortocoid activity of liquorice: 11-beta-hydroxy-steroid dehydrogenase deficiency comes of age", pages 820-824, see the whole article (cited in the application)	1,2

---

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22nd January 1990	20 FEB 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND incompletely searchable

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers * because they relate to subject matter not required to be searched by this Authority, namely:

* 3-6
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8901266 SA 32109

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 09/02/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 843135		None	
FR-M- 4666		None	
DE-A- 3443242	28-05-86	CH-A- 662510	15-10-87
		FR-A- 2573655	30-05-86
		GB-A,B 2167296	29-05-86
		IL-A- 65184	30-08-85

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82